Development of Microbial Electrochemical Cell for recovery of heavy metals from water of Damanganga River"

Synopsis to be Submitted to

Swami Ramanand Teerth Marathwada University, Nanded

For the research work leading to the award of the degree of

Doctor of Philosophy
In Microbiology
Under the faculty of Science and Technology

By
Mr. Shantanu S. Mishra
Researcher
M.Sc., SET .
(Temporary registration No: U4021AP15122020)
Mobile: +91 9904301084, **Mail Id:** shantanum2015@gmail.com

Category: OPEN

Under Guidance of

Dr. Deepak U. Bhusare
Research Supervisor
Assistant Professor
Department of Microbiology
D.B ACS College , Bhokar.
Swami Ramanand Teerth Marathwada University, Nanded
Mobile: +91 9404351333, **Mail Id:** bhusaredu@gmail.com

PET 2020

1. INTRODUCTION

Rapid and disorganized industrialization and urbanization have contributed to elevated levels of heavy metals in developing countries such as China and India's urban environments (Gupta et al., 2012).Untreated industrial waste water discharged into nearby bodies of water can lead to severe ground water pollution (Mustapha & Halimoon, 2015). Industrial wastewaters and sewage sludge applications have long-term toxic effects on humans and the environment (Kartik et al., 2016).

Heavy metals in industrial effluents are known to pose a serious risk to natural water, animal, and human health(Mustapha & Halimoon, 2015).Heavy metals like lead (Pb), chromium (Cr), cadmium (Cd), mercury (Hg), iron (Fe), cobalt (Co), and nickel (Ni) are significant environmental pollutants, notably in areas with high anthropogenic pressure. .Their presence, even in trace amounts, in the atmosphere, soil, and water can pose a threat for all organisms (Gupta et al., 2012) Heavy metals and their derivatives can be deposited on surfaces and then adsorbed by plant and animal tissues as they are non-biodegradable and persistent environmental contaminants. (Gupta et al., 2012) Plants absorb heavy metals from polluted environments as well as from contaminated water. Vegetables are commonly grown along the banks of rivers that run through urban areas in many developing countries. Heavy metals have frequently been reported to pollute the waters of such rivers. (Gupta et al., 2012)

Contaminants accumulating in soil, environment, and crops as a result of sewage water, fertilizer, and pollutants are a major concern. Even at very low concentrations, heavy metals are mutagenic, teratogenic, neurotoxic, and carcinogenic. Metal toxicity has also been linked to the development of several diseases in humans, including cardiovascular problems, tubular dysfunction in the kidneys, and nervous disorders. (Gupta et al., 2012) Mercury can combine with other elements to form organic and inorganic compounds. Adding it to the list of one of the most hazardous heavy metals. The toxicity of mercury in its three forms (elemental, organic, and inorganic compounds) varies. (Farahani et al., 2020) Various chemical forms of mercury exist in nature, including elemental mercury vapour (Hg0), inorganic mercurous (Hg+1), mercuric (Hg+2), and organic mercury compounds, and they all have an impact on human and animal health. Mercury leads to severe alterations in body tissues, resulting in negative health effects.

When people are exposed to high levels of mercury, they are very likely to suffer permanent brain, kidney, and fatal damage. Variety of lung and skin problem have been reported to be caused by Short-term exposure to high levels of mercury vapours. To avoid these consequences, the maximum allowed mercury levels in drinkable water and sea food are 2 and 1 ppm, respectively (Farahani et al., 2020)

Electrochemical treatment, ion exchange, evaporation, chemical. precipitation, reverse osmosis, and sorption are some of the traditional methods used to remove heavy metals from contaminated effluent. All of these physical and chemical methods, however, were found to have technical and economic limitations, such as high operating costs and the release of chemical and massive sludge into the environment as a by-product as a result, there is a need to replace them with more cost-effective and environmentally friendly biological treatments. (Mustapha & Halimoon, 2015)

Microbes can consume organic waste. When microorganisms consume waste, they transform it into nontoxic by products and during the conversion process, many metabolites are produced in order to degrade the complex waste into simple compounds. This is due to the fact that microorganisms have evolved numerous resistance mechanisms to survive in the presence of toxic heavy metals in their environment. Metal sorption, extracellular precipitation uptake and accumulation, mineralization and enzymatic oxidation or reduction to a non-toxic form, and efflux of heavy metals from the cell are among the strategies developed by microbes. (Mustapha & Halimoon, 2015)

Exploring microbial habitats with high levels of heavy metals has major implications for microbial heavy metal tolerance because a heavy metal's oxidation state is related to its solubility and toxicity. Microorganisms' metal accumulating capacity can be used to remove, concentrate, and recover metals from industrial effluents and mine tailings. There have been several reports of aerobic bacteria accumulating metals such as Ag, Co, Cu, Cr, and Ni. Bioremediation, as Microorganism-based remediation technique, have the potential to degrade and detoxify certain contaminants. (Mustapha & Halimoon, 2015)

The Damanganga River originates from Sahyadri Hills near Valveri village in Nashik district of Maharashtra. The river flows 131.30 kilometers from east to west, passing through the hilly areas of Maharashtra, Gujarat, and the Union Territories (UT) of Dadra & Nagar Haveli

(DNH) and Daman & Diu (DD) before draining into the Arabian Sea. The major water resource is the Madhuban Dam, which was built upstream of the river in Gujarat state. The river runs 41.56 kilometers from Madhuban Dam to its confluence with the Arabian Sea. There are approximately 5105 larges, medium, and small-scale industries located along the river's stretch in Dadra & Nagar Haveli, Vapi (Gujarat), and Daman industrial clusters. Vapi GIDC in Gujarat's District Valsad is one of Asia's largest industrial areas. Fine chemicals, pharmaceuticals, dyes, dye intermediates, textiles, pesticides, agrochemicals, various metals, alloys, and many other industries are included in it. The remainder consists primarily of pulp and paper mills, plastics, and food processing units. The Central Pollution Control Board (CPCB) released a list of critically polluted areas in December 2009 using a comprehensive environmental pollution index (CEPI) .Vapi GIDC was the second most polluted industrial area in India, with a CEPI index of 88.29, and Ankleshwar was the first, with a CEPI index of 88.5. . (Nirgude et al., 2013.) Most industries do not provide primary treatment for their own industrial effluents, resulting in large amounts of effluent containing unacceptably high levels of pollutants. A large amount of partially treated and highly polluted effluent is discharged into the Damanganga. (Nirgude et al., 2013.) Domestic wastewater is also discharged into the river from the catchment area. The major sources of wastewater discharges into Damanganga River through various drains include the disposal of industrial effluents from the industrial units/clusters in DNH, Vapi (Gujarat), and Daman as well as domestic sewage from towns/cities and settlements along the bank of the river. The most significant industrial effluents discharged into the river and its estuary are from the Common Effluent Treatment Plant (CETP) of the GIDC, Vapi and Gujarat Heavy Chemicals Limited (GHCL), Bhilad. The treated effluent from the CETP, Vapi is discharged into the river downstream of the Vapi check dam. The river water downstream of the CETP's Vapi discharge point is highly coloured, indicating severe pollution, which lasts until the river's confluence with the Arabian Sea.

Fig: Heavy metal containing hazardous effluent discharge in to the river

The aims of this research is to isolate and characterize bacteria from Damanganga water which receives effluents from CETP'S, industries as well as domestic sewage. The all year round hot and humid climate makes this site an incubator for a wide range of microbes. The water flows in the area are strongly impeded because of steep population growth and urbanization along its length. Most of the people living in this area are poor and therefore use this as a household water source. The main source of food for these people is the farming practice on the ground around the canal. The site was therefore chosen to isolate and characterize variety of metal-tolerant bacteria and asses the resistance and bioremediation potential of those bacteria to different heavy metals.

2. REVIEW OF LITERATURE AND DEVELOPMENT IN THE SUBJECT

2.1 Effect of heavy metal Pollution

The impact of various industrial activities on heavy metal concentrations. They found that The expansion of industrial activity in recent years has increased pollution in this environment, causing health problems for workers and the surrounding community (Gabarrón et al., 2017). Lin et al., reported that heavy metals exposure during pregnancy is a significant risk factor for adverse birth outcome (Lin et al., 2018).

2.2 Status of Trace and Toxic Metals in Indian Rivers

Pollution caused by heavy metals in aquatic environments is a growing problem in India as well as around the world, and it has reached an alarming level. Recently (Hussain et al., 2017) collected Godavari river basin Surface water samples and used atomic absorption spectrophotometer to quantify the concentration of eight heavy metals such as arsenic, cadmium, chromium, copper, iron, lead, nickel, and zinc. The data analysis revealed that out of 18 water quality stations monitored 12 stations were above the desirable limit due to presence of copper and nickel metals. (Sharma et al., 2020) reported that, Yamuna river water and surrounding soil are tainted with a variety of pollutants, including heavy metals. They found Heavy metal concentrations were higher than their safe limit at all ten sampling points. Similarly heavy metal contamination has been reported in Hindon River , (S. Mishra et al., 2021) , Gomti River (Gaur et al., 2005) . (Patel & Sahoo, 2021) conducted seasonal investigation of the water quality of the Damanganga estuary from two riverine points (downstream and upstream) From January to

December 2019. The WQI (Water Quality Index) indicates that the estuarine water quality has deteriorated due to the high value of heavy metal presence (Aluminium, Iron, Manganese, Boron, and Zinc), as well as increased contents of Chloride, Ammonium, and Sulphate in riverine water. (Nirgude et al., 2013) Did physicochemical analysis of Vapi ground water samples collected from various sources which showed the impact of industrial effluent pollution on ground water. All of the physicochemical parameters like pH, Colour, EC, TDS, TH, BOD, COD, DO, Cl-,SO4-2, Na+1,K+1 Ca+2, Mg+2 were well above the WHO and USEPA drinking water permissible limits. River Data Compilation -2, 2018 report from Ministry of Water Resource, Central Water Commission, and Government of India indicate that Damanganga river water has exceeded the acceptable limit for lead, iron and copper.

2.3 Isolation of heavy metal resistant bacteria

The isolated eight nickel resistant bacteria from wastewaters polluted with different industrial sources (Hoda et al., 2014). Based on their Maximum tolerable concentrations (8, 16, and 24 mM Ni2+), three of them were chosen as the most resistant which were identified as *Cupriavidus* sp ATHA3, *Klebsiella oxytoca* ATHA6 and *Methylobacterium* sp ATHA7 by 16SrDNA gene sequencing. Their research also revealed that after three days, K oxytoca ATHA6 could remove 83 mg/ml of nickel from the medium. (Mustapha & Halimoon, 2015) screened Heavy metals tolerant bacteria from electroplating industry. They tested 21 colonies in the presence of various heavy metals and discovered that 5 isolates could tolerate 50 mg/l of cr, cu, pb, and cd. . Two isolates were tolerable to 200mg/lit cadmium, while one was highly resistant to copper. (Osman et al., 2010) isolated strain belonging to genus Halomonas from the polluted Maruit Lake, Egypt which showed high copper resistance up to a concentration of 1.5 mMol /l) was also resistant to other metals like nickel, cobalt, and zinc. Majority of the copper accumulated in the cells' outer membrane and periplasmic fractions as revealed by Cell fractionation and electron microscopy. It was also found that chromate, cobalt, zinc and cadmium resistance was plasmid conferred. (Farahani et al., 2020) studied bio removal of nickel and mercury using iron oxidising bacteria isolated from different sources. Their study showed 100% of the mercury and maximum of 30% nickel war removed by bacterial culture which was identified as *Bacillus velezensis* CR-502. Kartik et al., 2016) isolated and identified eleven potential Cd resistant bacteria from soils of Damanganga river which belonged to 4

different genera such as *Providencia* spp, *Morganella* sp, *Stenotrophomonas* sp. and *Bacillus* spp. Rajkumar et al., 2012, isolated 4 chromium tolerant bacteria from Barak River Contaminated with Pulp Paper Mill Effluent, South Assam (Rajkumar et al., 2012).

2.4 Bio removal/ biosorption of heavy metals

Recently, the removal of different heavy metals by various bacteria has been reported and Bacteria have been also reported as effective heavy metal bio sorbents .(Saranya et al., 2018) investigated the potential detoxification efficiency of heavy metals by bacteria isolated from coral, sea grass, and mangrove environments . The percentages of metal removal by bacteria in experiments with a 100 mg /l initial metal concentration were 55.23 percent for Cd, 72.45 percent for Cr, 76.51 percent for Cu, and 61.51 percent for Zn, respectively. Furthermore, heavy metal biosorption was confirmed using Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM). (Bachate et al., 2013) showed that *Bacillus firmus* TE7 cells reduced Cr (VI) and oxidized As (III) simultaneously and independently. The active uptake of metals was proposed as a mechanism for metal removal. Arsenic oxidation was discovered to be caused by the arsenite oxidase enzyme. They also found that The conversion of Cr(VI) to Cr (III) was accomplished through enzymatic reduction . (Poirier et al., 2013) described the bio removal of Cd, Zn, and Cu during the growth of the marine bacterium Pseudomonas fluorescens BA3SM1. Extracellular metal biosorption and intracellular metal uptake have been proposed as Metal removal mechanisms. They also performed global proteomic evaluation of response of bacteria against heavy metal by two-dimensional gel electrophoresis and mass spectrometry which showed that the cell adapts to metals by activating seven distinct defence mechanisms Cell aggregation/biofilm formation (Zn = Cu > Cd); envelope modification to increase extracellular metal biosorption and/or control metal uptake (Cu > Zn); metal export (Cd = Zn and probably Cu); oxidative stress responses (Cu > Zn > Cd); intracellular metal sequestration (Zn = Cu and probably Cd); hydrolysis of abnormally folded proteins (Cd = Cu); and over-synthesizing. Fan et al.(2014) reported passive uptake of Cu and Cr by *Ochrobactrum intermedium* LBr and *Cupriavidus metallidurans* CH34 .The cell wall's negatively charged functional groups were responsible for the binding of positively charged metal ions. (Bueno et al., 2008) used SEM analysis to investigate the biosorption of Cr(III), Cu(II), and Pb(II) by *Rhodococcus opacus*. The nature of potential cell–metal ion interactions was also investigated using FT-IR and

SEM/EDS. These investigations revealed that the –COOH, –OH, and –NH groups were involved in the biosorption process. The removal of Zn(II) and Cd(II) by biosorption using Undaria pinnatifida was investigated in batch and dynamic systems by (Plaza Cazón et al., 2013). According to SEM-EDX analysis, the active sites are distributed unevenly on the cell wall surface.(Das et al., 2014) did Investigation on mechanism of Cr(VI) reduction and removal by *Bacillus amyloliquefaciens* isolated from chromite mine soil of Sukinda, India. The strain had a relatively high Cr (VI) tolerance (900 mg /L)) and a fast reduction rate of 2.22 mg Cr(VI) /L /h. The SEM image of the bacterial cell before biosorption was found to be flat with a nonporous structure at lower magnification and a regular, smooth surface at higher magnification. This demonstrated the consistency of the bacterial cell surfaces. The cell surface was found to be uneven and porous at lower magnification and irregular at higher magnification after Cr(VI) biosorption. In the SEM micrograph of the cells after Cr(VI) biosorption, amorphous substances were found all over the cell surface. This indicates that Cr(III) precipitation in the form of round globules has occurred. The TEM–EDX analysis of the bacterial precipitates after Cr(VI) treatment revealed intracellular deposition of Cr(III) in the nanometric range. They suggested both the surface immobilization and intracellular accumulation of Cr(III) along with the formation of coagulated cell precipitate by living *B. Amyloliquefaciens*. (Prithviraj et al., 2014) also studied Biosorption of nickel by *Lysinibacillus sp*. SEM-EDX confirmed Ni(II) adsorption on live biomass, where cell aggregation and increasing irregularity of cell morphology were observed even though cells were in a non-growing state. . (Srivastava & Thakur, 2007), (Dhal et al., 2010)(R. R. Mishra et al., 2012). used Transmission electron microscopic (TEM) analysis to examine the inner structure of the cells before and after biosorption to locate the intracellular accumulation of metals .(Kiliç et al., 2010) used proteomic approach identify proteins involved in Cr(VI) stress response of *Pseudomonas aeruginosa* to toxic Cr(VI) they used MALDI–TOF MS and LC–MS/MS to identify proteins whose expression level increased or decreased after exposure to Cr(VI). (Thompson et al., 2010) discovered that Cr(VI) stress increased the synthesis of enzymes involved in amino acid and protein metabolism using LC-MS analysis. HPLC was used to study metabolites after biodegradation of metal complex dyes by (Kalpana et al., 2011) . They found new peaks, suggesting the formation of new metabolites. The disappearance of other peaks indicated the removal of some compounds following Cr complex dye bioremediation. To determine the various changes in functional groups in the solution before and after biosorption,

Fourier transform infrared spectroscopic analysis is used. The presence of carboxyl, hydroxyl, and amino groups in biomass was revealed by FTIR analysis, which appear to be responsible for Ni biosorption (II) (Prithviraj et al., 2014) and for biosorption of Cr(III), Cu(II), and Pb(II) (Bueno et al., 2008). According to FT-IR spectrometry analysis (Plaza Cazón et al., 2013) found that Carboxylic groups and chemical groups containing N and S contribute to Zn(II) and Cd(II) uptake by *U. Pinnatifida*.

2.5 Recovery of heavy metal by MEC

Recently, waste water treatment using biofuel technology has gained popularity due to its bio-sustainable resource of powering microbes (electrical energy), which exponentially reduces reliance on fossil fuels. Microbial electrolysis cells (MECs) have been developed in the last decade to treat waste water and produce energy. It is regarded as a potential green technology for addressing the issues of energy scarcity and global warming (Anwer et al., 2020) (Jiang et al., 2020) proposed a novel microbial metals enrichment and recovery cell (MMERC) for efficient and cost-effective recovery of Cu(II) and Cd(II) at low concentrations. Cu(II) and Cd(II) concentrations in contaminated wastewater are reduced from 5 mg/ L to less than 0.2 mg/ L during the operation, and these separated metals are highly enriched in the catholyte and removed simultaneously with 99.0 percent for Cu(II) and 96.5 percent for Cd(II).

3. OBJECTIVES OF RESEARCH:

3.1 Physico-chemical analysis of Damanganga River water.

3.2 Screening and characterisation of heavy metal resistant bacteria form the Damanganga River Water.

3.3 To study the heavy metal resistance and bioremediation ability of isolate.

3.4 Identification of isolates showing significant heavy metal resistance and bioremediation ability by 16S rRNA gene sequencing.

3.5 In silico analysis of the identified cultures through Bioinformatics tools.

3.6 Genomic investigation whether the isolates, heavy metal resistance is Plasmid-mediated.

3.7 Study the mechanism of metal removal like bioaccumulation/ biosorption/enzymatic degradation by selected isolates using various analytical techniques.

3.8 Statistical analysis of bioremediation by the isolate.

3.9 Recovery of heavy metals from waste water using microbial electrochemical technologies like Microbial Electrochemical Cells (MEC)

.

4. METHODS AND MATERIALS

4.1 Study area-

The stretch of Damanganga River starting from Madhuban Dam and passing from towns of Silvassa, Vapi and Daman till its confluence with Arabian Sea at Daman.

4.2 Water sample collection and sampling points –

Water samples will be collected in clean, dry and acid soaked polythene container form

a. various major industrial effluents discharge points along the Damanganga river stretch from Silvassa, vapi and Daman
b. Treated effluent Discharge point from CETP vapi.
c. Treated effluent discharge point from ETP of Gujarat Heavy Chemical Limited (GHCL), Bhilad, Vapi, Gujarat

4.3 Physico-chemical analysis of Damanganga River Water

Physico chemical analysis of collected water samples will be done using standard procedures prescribed in American Public Health Association (APHA) and Concentration of different heavy metals will be determined by using atomic absorption spectrometer.

4.4 Screening and characterisation of heavy metal resistant bacteria

Water sample collected from various discharge points will be serially diluted and different dilutions will be spread on Nutrient agar/LB agar plates containing (20mg/l) of different heavy metals salts like $Pb(NO3)2$, $NiSO4 \cdot 6H2O$, $HgCl2$, $K2Cr2O7$, $CoCl2 \cdot 6H2O$,$CdCl3$, and $FeSO4 \cdot 7H2O$. After incubation for 24-48 hrs colonies showing different morphological characters will be selected for further analysis. Biochemical characterisation of isolates will be done by test according to Bergey's Manual of Systematic Bacteriology.

4.5 Determination of Heavy metal resistance and bioremediation ability

The maximum resistance of the isolated bacteria will be determined against increasing concentrations of different heavy metals on nutrient agar plates until the strains unable to grow on the agar plates. The initial concentration of these metal salts in nutrient plates will be 50 µg/mL, and bacterial growth will be observed by streaking on respective plates. Metal salt concentration will be progressively increased by 10–15 µg/ml on a fresh agar plate, and the Minimum Inhibitory Concentration (MIC) or Maximum Tolerable Concentration (MTC) will be noted when the isolates will fail to grow on respective plates. The experiment will be conducted separately for different heavy metal.

Heavy metal removal rates by the tested isolates will be determined following their incubation N.B/L.B medium containing heavy metal at conc. of 100 ug/ml and measurement of residual heavy metal in culture supernatant. At selected intervals of time samples will be harvested and centrifuged, supernatant will be used to determine residual heavy metal concentration.

4.6 Identification of isolates by 16S rDNA sequencing

Genomic DNA will be extracted from isolates showing maximum heavy metal resistance and bioremediation ability. Bacterial 16S rDNA Will be amplified from the extracted genomic DNA by Polymerase chain reaction using the universal bacterial 16S rDNA primers. PCR products will be analysed by electrophoresis in 1.5% (w/v) agarose gel in 1x TAE buffer with ethidium bromide and then will be subjected to DNA sequencing. The sequences from each isolate will be used as queries in GenBank database to determine the genus and species

4.7 Genomic investigation of Heavy Metal Resistance

To see weather heavy metal resistance is chromosomally encoded or plasmid mediated, Isolation and detection of plasmid DNA will be done by using Alkaline lysis method / by Himedia plasmid isolation kit followed by agarose gel electrophoresis. If isolates show presence of Plasmid then Curing of Plasmid DNA will be done using acridine Orange or Mitomycin C. Cured isolates will be then subjected to growth on Nutrient media containing Heavy Metal to check whether they still retain heavy metal resistance or not.

4.8 Determination of mechanism of Metal Removal by isolates

Biosorption of Heavy metals by the isolates can be studied by Fourier transform infrared spectroscopy (FT-IR), Scanning electron microscopy (SEM-EDX) analysis. Transmission electron microscopy (TEM) can be used to study intracellular uptake, deposition or accumulation of heavy metals by the isolates. Changes in protein profile of isolates in response to heavy metals can be studied using MALDI–TOF MS and LC–MS/MS. To study metabolites after biodegradation of heavy metal and its bio removal, HPLC can be used.

4.9. Recovery of heavy metals

Recovery of heavy metal can be done by construction of Microbial Electrochemical Cell (MEC).

4.10 Statistical analysis

All the results will be tested statistically by analysis of variance (ANOVA) using the SPSS software version, with P values of <0.05 as the level of significance.

5. IMPORTANCE OF STUDY/ SOCIETY APPLICATION

5.1 In waste water treatment plants –

Treatment of industrial waste (Pharmaceutical, chemical, pesticide, dye) as well as CETP (Common effluent treatment plant) waste containing heavy metals by isolates may be appropriate solution for effluent disposal problems.

5.2 In Agriculture –

Clean-up of heavy metal polluted agricultural soils and the recovery of plant growth promoting and nitrogen cycle microbial communities

5.3 Bio augmentation and Bio stimulation –

Using isolates effect of Bio augmentation and Bio stimulation can be studied to restore /support the growth of natural inhabitants

5.4 Recovery of heavy metals and energy production

6. PROPOSED WORK PLAN

Year-wise Plan of work and targets to be achieved

6.1 First Year:

6.1.1. Reference/Literature Collection:

6.1.2. Collection of water sample from Damanganga River:

To collect the Water samples various major industrial effluents/CETP discharge points along the Damanganga river stretch from Silvassa, vapi and Daman in a sterile condition to minimize the local contamination and bring in the laboratory for further procedure.

6.1.3. Physico-chemical Characterization of water:

The collected water sample should be analyzed for their physico-chemical contents to know the pollution level and concentration of heavy metals.

6.1.4. Screening and Isolation of heavy metal resistant bacteria:

Heavy metal resistant bacteria should be Screened and isolated from the collected water sample.

6.1.5. Physico-chemical characterization of isolated strains:

The biochemical characterization should be compulsorily done for tentative identification of microorganisms.

6.1.6 Submission of Progress Report

6.1.7 Preparation of Research Paper

6.2 Second Year:

6.2.1. Determination of Heavy metal resistance:

Heavy metal resistance of the isolates should be determined to find suitable candidate for bio removal and bioremediation of heavy metals.

6.2.3 Determination of bioremediation ability of Isolates

Isolates showing significant heavy metal resistance should be checked for their bioremediation ability.

6.2.4 Identification of isolated bacterial strain by 16S-rRNA sequencing:

The tentatively identified species showing significant Heavy metal resistance and bioremediation ability should be identified by 16S rDNA sequencing for species identification.

6.2.5 Genomic investigation of Heavy Metal Resistance:

Isolation, detection and curing of plasmid DNA should be done to determine chromosomal or extrachromosomal origin of heavy metal resistance,

6.2.6 Study mechanism of Metal Removal by isolates

Mechanism of heavy metal removal like Biosorption, intracellular uptake, deposition or accumulation, and Changes in protein profile should be studied by using various analytical techniques.

6.2.6 Submission of Progress Report

6.2.7 Preparation of Research Paper

6.3 Third Year:

6.3.1 Study of metabolites formed after Bioremediation:

Metabolites formed after bioremediation should be studied to find end products of heavy metal degradation.

6.3.2 Recovery of heavy metals

Recovery of heavy metals should be done by construction of Microbial Electrochemical Cell (MEC).

6.3.4. Use of SPSS Software

6.3.5 Preparation of Graphs

6.3.6 Data Interpretation

6.3.7 Final Compilation of Data

6.3.8 Preparation of Research Paper & Communicating it for Publication

6.3.9 Report Writing

6.3.10 Submission of Final Report to University with all details.

7. REFERENCE

Anwer, A.H. *et al.* (2020) 'Microbial Electrochemical Cell: An Emerging Technology for Waste Water Treatment and Carbon Sequestration', in *Modern Age Waste Water Problems*. Springer International Publishing, pp. 339–360. doi:10.1007/978-3-030-08283-3_17.

Bachate, S.P. *et al.* (2013) 'Simultaneous reduction of Cr(VI) and oxidation of As(III) by Bacillus firmus TE7 isolated from tannery effluent', *Chemosphere*, 90(8), pp. 2273–2278. doi:10.1016/j.chemosphere.2012.10.081.

Bueno, B.Y.M. *et al.* (2008) 'Biosorption of lead(II), chromium(III) and copper(II) by R. opacus: Equilibrium and kinetic studies', *Minerals Engineering*, 21(1), pp. 65–75. doi:10.1016/j.mineng.2007.08.013.

Das, S. *et al.* (2014) 'Investigation on mechanism of Cr(VI) reduction and removal by Bacillus amyloliquefaciens, a novel chromate tolerant bacterium isolated from chromite mine soil', *Chemosphere*, 96, pp. 112–121. doi:10.1016/j.chemosphere.2013.08.080.

Dhal, B. *et al.* (2010) 'Reduction of hexavalent chromium by Bacillus sp. isolated from chromite mine soils and characterization of reduced product', *Journal of Chemical Technology and Biotechnology*, 85(11), pp. 1471–1479. doi:10.1002/jctb.2451.

Farahani, S. *et al.* (2020) 'Bio-removal of heavy metalsusing iron-oxidizing bacteria: A novel approach in environmental biotechnology', *Iranian Journal of Pharmaceutical Research*, 19(3), pp. 421–429. doi:10.22037/ijpr.2019.112474.13779.

Gabarrón, M., Faz, A. and Acosta, J.A. (2017) 'Effect of different industrial activities on heavy metal concentrations and chemical distribution in topsoil and road dust', *Environmental Earth*

Sciences, 76(3). doi:10.1007/s12665-017-6449-4.

Gaur, V.K. *et al.* (2005) 'Distribution of heavy metals in sediment and water of river Gomti', *Environmental Monitoring and Assessment*, 102(1–3), pp. 419–433. doi:10.1007/s10661-005-6395-6.

Gupta, K., Chatterjee, C. and Gupta, B. (2012) 'Isolation and characterization of heavy metal tolerant Gram-positive bacteria with bioremedial properties from municipal waste rich soil of Kestopur canal (Kolkata), West Bengal, India', *Biologia*, 67(5), pp. 827–836. doi:10.2478/s11756-012-0099-5.

Hussain, J. *et al.* (2017) 'Studies on heavy metal contamination in Godavari river basin', *Applied Water Science*, 7(8), pp. 4539–4548. doi:10.1007/s13201-017-0607-4.

Jiang, Q. *et al.* (2020) 'In-situ enrichment and removal of Cu(II) and Cd(II) from low-strength wastewater by a novel microbial metals enrichment and recovery cell (MMERC)', *Journal of Power Sources*, 451. doi:10.1016/j.jpowsour.2019.227627.

Kalpana, D. *et al.* (2011) 'Bioremediation of the heavy metal complex dye Isolan Dark Blue 2SGL-01 by white rot fungus Irpex lacteus', *Journal of Hazardous Materials*, 198, pp. 198–205. doi:10.1016/j.jhazmat.2011.10.030.

Kartik, V.P., Jinal, H.N. and Amaresan, N. (2016) 'Characterization of cadmium-resistant bacteria for its potential in promoting plant growth and cadmium accumulation in Sesbania bispinosa root', *International Journal of Phytoremediation*, 18(11), pp. 1061–1066. doi:10.1080/15226514.2016.1183576.

Kiliç, N.K. *et al.* (2010) 'Proteomic changes in response to chromium(VI) toxicity in Pseudomonas aeruginosa', *Bioresource Technology*, 101(7), pp. 2134–2140. doi:10.1016/j.biortech.2009.11.008.

Lin, Z. *et al.* (2018) 'Individual heavy metal exposure and birth outcomes in Shenqiu county along the Huai River Basin in China', *Toxicology Research*, 7(3), pp. 444–453. doi:10.1039/c8tx00009c.

Mishra, R.R. *et al.* (2012) 'Optimization and characterization of chromium(VI) reduction in saline condition by moderately halophilic Vigribacillus sp. isolated from mangrove soil of Bhitarkanika, India', *Journal of Hazardous Materials*, 227–228, pp. 219–226.

doi:10.1016/j.jhazmat.2012.05.063.

Mishra, S., Kumar, A. and Shukla, P. (2021) 'Estimation of heavy metal contamination in the Hindon River, India: an environmetric approach', *Applied Water Science*, 11(1). doi:10.1007/s13201-020-01331-y.

Mustapha, M.U. and Halimoon, N. (2015) 'Screening and Isolation of Heavy Metal Tolerant Bacteria in Industrial Effluent', *Procedia Environmental Sciences*, 30, pp. 33–37. doi:10.1016/j.proenv.2015.10.006.

Nirgude, N.T., Shukla, S. and Venkatachalam, A. (no date) 'Physico-Chemical Characteristics And Quality Assessment Of Some Ground Water Samples From Vapi Town, Gujarat, India', 6(1), pp. 47–51.

Osman, O. *et al.* (2010) 'Copper-resistant halophilic bacterium isolated from the polluted Maruit Lake, Egypt', *Journal of Applied Microbiology*, 108(4), pp. 1459–1470. doi:10.1111/j.1365-2672.2009.04574.x.

Patel, S.S. and Sahoo, S. (2021) 'Investigation of water quality from Damanganga estuary the water quality indexing, Gujarat', *Sustainability, Agri, Food and Environmental Research* [Preprint]. doi:10.7770/safer-v0n0-art2356.

Plaza Cazón, J. *et al.* (2013) 'Zinc and cadmium removal by biosorption on Undaria pinnatifida in batch and continuous processes', *Journal of Environmental Management*, 129, pp. 423–434. doi:10.1016/j.jenvman.2013.07.011.

Poirier, I. *et al.* (2013) 'Strategies developed by the marine bacterium Pseudomonas fluorescens BA3SM1 to resist metals: A proteome analysis', *Aquatic Toxicology*, 128–129, pp. 215–232. doi:10.1016/j.aquatox.2012.12.006.

Prithviraj, D. *et al.* (2014) 'Biosorption of nickel by Lysinibacillus sp. BA2 native to bauxite mine', *Ecotoxicology and Environmental Safety*, 107, pp. 260–268. doi:10.1016/j.ecoenv.2014.06.009.

Rajkumar, B., Sharma, G.D. and Paul, A.K. (2012) 'Isolation and characterization of heavy metal resistant bacteria from barak river contaminated with Pulp paper mill effluent, South

Assam', *Bulletin of Environmental Contamination and Toxicology*, 89(2), pp. 263–268. doi:10.1007/s00128-012-0675-y.

Saranya, K. *et al.* (2018) 'Biosorption of multi-heavy metals by coral associated phosphate solubilising bacteria Cronobacter muytjensii KSCAS2', *Journal of Environmental Management*, 222, pp. 396–401. doi:10.1016/j.jenvman.2018.05.083.

Sharma, R., Singh, N.S. and Singh, D.K. (2020) 'Impact of heavy metal contamination and seasonal variations on enzyme's activity of Yamuna river soil in Delhi and NCR', *Applied Water Science*, 10(3). doi:10.1007/s13201-020-1166-7.

Srivastava, S. and Thakur, I.S. (2007) 'Evaluation of biosorption potency of Acinetobacter sp. for removal of hexavalent chromium from tannery effluent', *Biodegradation*, 18(5), pp. 637–646. doi:10.1007/s10532-006-9096-0.

Thompson, D.K. *et al.* (2010) *Proteomics reveals a core molecular response of Pseudomonas putida F1 to acute chromate challenge*, *BMC Genomics*.

Mr. Shantanu S. Mishra
Research Scholar

Dr. Deepak U. Bhusare
Research Guide